Peer Review
Experimental Toxicology Division
N H E E R L

Potential Allergenicity of Low Molecular Weight (LMW) Chemicals

SUSCEPTIBILITY, ALLERGY & ASTHMA

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INTRODUCTION

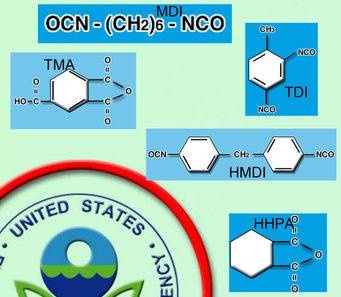
Exposure to certain low molecular weight (LMW) compounds (haptens) is associated with both dermal and respiratory hypersensitivity. The resulting diseases include occupational asthma (OA) and allergic contact dermatitis (ACD). The local lymph node assay (LLNA) has been adopted by regulatory agencies to screen chemicals for ACD, but there is no well validated test to identify chemicals with the potential to induce OA. However, OA inducing chemicals are thought to be a subset of chemicals that produce ACD (positive LLNA response). EPA's pre-manufacturing notice program needs a cost-effective screen to identify LMW chemicals that produce OA.

OBJECTIVES

1. Determine whether serum total IgE or profiling cytokines from the draining lymph nodes following dermal exposure will be useful for distinguishing chemical asthmagens (OA) from other LLNA positive chemicals

2. Using a mouse model explore alternative exposure regimens and endpoints that might be useful in identifying OA chemicals

Representative diisocyanate and acid anhydride chemicals of concern



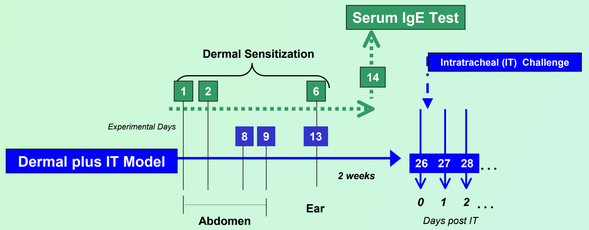
METHODS AND APPROACH

Two experimental approaches:

1. TMA (associated with OA in humans) vs. DNCB or DNFB (not associated with OA) (Figures 1 & 2)

Mouse/Serum IgE Test: An interlaboratory evaluation of serum total IgE levels following repeated dermal exposure as a means to distinguish OA from non-OA chemicals.

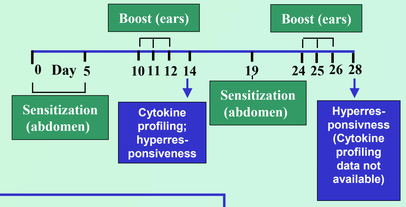
Dermal plus Intratracheal (IT) Exposure Model: After dermal sensitization, IT challenge with LMW chemicals (either protein-conjugated or free), endpoints typical of OA were assessed.



2. Differential responses of various acid anhydrides and diisocyanates (Figures 3,4,& 5)

RNase Protection Assay (RPA) Cytokine Profiling: Evaluation of cytokines from the draining lymph node of mice following dermal exposure (protocol below). Isocyanate data are shown.

Pulmonary Hyperresponsiveness to Methacholine Challenge : Mice were treated with isocyanates using the exposure protocol below. Hyperresponsiveness was assessed using whole body plethysmography (Buxco). Goal: Relate cytokine profiles to respiratory responses and develop a correlation to OA.

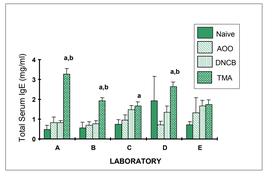


LMW Chemical Abbreviations:

TMA: trimellitic anhydride
DNFB: dinitrofluorobenzene
DNCB: dinitrochlorobenzene
TDI: toluene diisocyanate
MDI: diphenylmethane-4,4'-diisocyanate
HMDI: dicyclohexylmethane-4,4'diisocyanate
IDPI: isophorone diisocyanate
TMI: P-tolyl (mono) isocyanate
TMXDI: meta-tetramethylene xylene diisocyanate

RESULTS

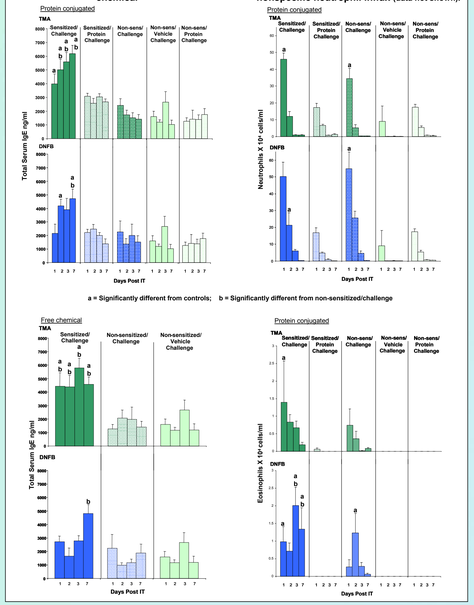
1 Serum IgE Test: Differences between TMA & DNCB, TMA & naïve, and TMA & vehicle were not consistent across laboratories. Background IgE variability associated with inconsistent responses.



An interlaboratory comparison of the mouse Serum IgE Test.
BALB/c mice were exposed (dermally) in each laboratory to DNCB, TMA, vehicle (AOO) alone or nothing (naïve).
a = significant increase in total serum IgE compared to vehicle-treated controls
b = significant increase in total serum IgE compared to DNCB treatment

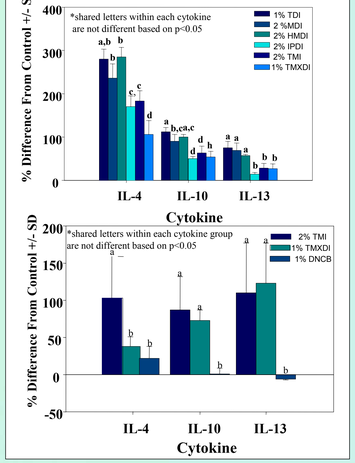
2 Significant IgE increases occurred in both TMA & DNFB sensitized mice after challenge with either protein-conjugated or free chemical

BALF cell influx following protein-conjugated DNFB or TMA depicts nonspecific inflammation. Likewise, free chemical elicits a nonspecific neutrophil influx (data not shown).

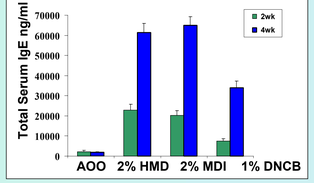


a = Significantly different from controls; b = Significantly different from non-sensitized/challenge

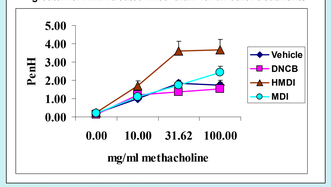
3 Cytokine Profiling: The Th2 cytokine signal for TMI &TMXDI was significantly greater than DNCB but significantly less than MDI & TDI



4 Total serum IgE increased following dermal exposure to isocyanates as well as DNCB



5 At 28 days hyperreactivity to methacholine was significantly greater for HMDI treated mice than for all other treatments



CONCLUSION

Serum total IgE increased following DNCB or DNFB exposure - 3 potential interpretations:
- IgE is not a good marker for OA
- DNCB/DNFB are poor "negative" controls
- LMW chemicals do not exhibit clear or distinct features of OA and ACD

The isocyanates tested produced a greater Th2 cytokine signal than DNCB using RNAse protection assay.

TDI and MDI (OA associated) gave a stronger signal than TMI and TMXDI (unknowns).

An animal model is needed to correlate the Th2 signal with respiratory effects.

Preliminary work showed enhanced hyperresponsiveness to methacholine following dermal exposure to HMDI, but not to MDI.

IMPACT

Currently, no test method can clearly distinguished LMW OA chemicals from other contact sensitizers. Additionally, chemicals that elicit ACD (an immune response) could also be detrimental to the lung (whether Th1, Th2, or both). Dermal and inhalation exposure to chemicals with a positive LLNA response should be avoided. These recommendations will be provided to EPA's Program Offices (OPPTS).

FUTURE DIRECTION

- Test all 6 isocyanates for induction of methacholine hyperreactivity & relationship to cytokine profiles and IgE responses.
- Evaluate oxazalone as an alternate "negative" control to this study.
- Shift in Agency priorities will redirect our allergy research program. Time and resources will be shifted away from this project in order to accommodate urgent needs in the area of food allergy

SOLVING AGENCY PROBLEMS